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Cellular Uptake of DNA Block Copolymer Micelles with Different Shapes

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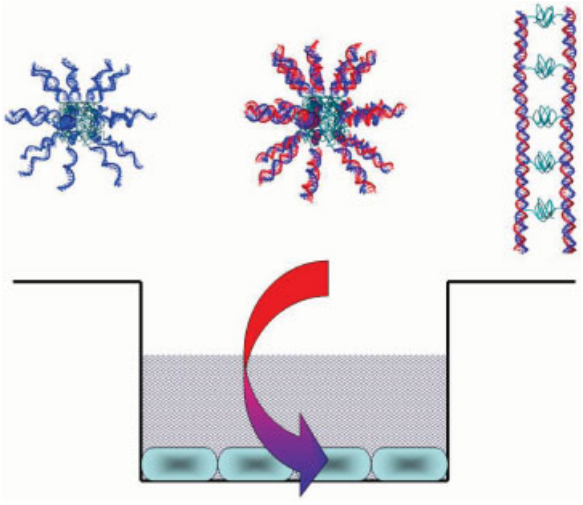
The cellular uptake of DNA block copolymer micelles composed of DNA-*b*-PPO in Caco-2 cells was studied. In particular it was investigated if the shape of micelle aggregates influences the internalization. Rod-like polymeric particles were taken up 12 times more efficiently than their spherical counter parts although they were composed of the same constituents. Furthermore, it was observed that internalization of all the micelle systems was more efficient than the pristine DNA controls. A cytotoxicity assay proved the non-toxic nature of DNA-*b*-PPO micelle aggregates.



Introduction

The cellular uptake of particles with sizes in the regime of nanometers is of great importance for two reasons. First, in biomedicine such particles have great potential for the delivery of therapeutics or being the carrier of imaging reagents. Second, with nanoparticles becoming incorporated into commercial products concerns are arising about their toxicity and their influence on living matter. In the context of organic nanoparticle fabrication, polymers play an important role. Representatives of this class of materials are dendrimers,[1,2] polymer lattices,[3] and block copolymers.[4] When latter ones consist of hydrophilic and hydrophobic segments these materials usually tend to form spherical micelles in aqueous solutions and thus can be regarded as nanoparticles. Recently, a special type of amphiphilic block copolymers with DNA as a water soluble segment and a hydrophobic organic polymer unit were introduced.[5,6] The resulting spherical aggregates with a shell of single stranded (ss) DNA were employed to deliver antisense oligonucleotides,[7] to produce binary assemblies with DNA-coated Au-nanoparticles[8] and to act as programmable scaffolds for DNA-templated organic

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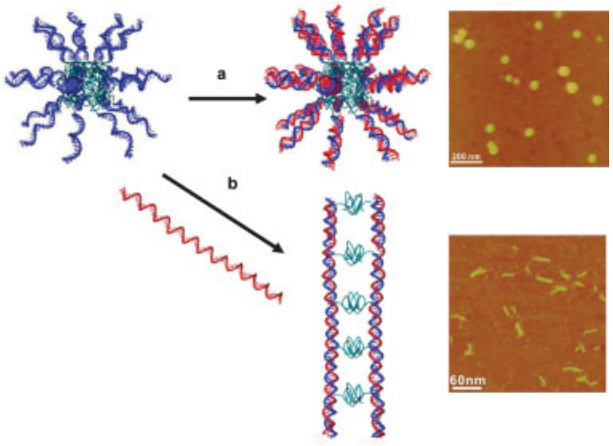

Figure 1. Preparation of nanoparticles with different shapes and corresponding AFM pictures of the resulting nanoobjects.[10] (a) Base pairing of ss DNA-b-PPO micelles with a short complementary sequence yields micelles with a ds corona maintaining the overall spherical shape of the aggregates. (b) Hybridization with long DNA templates results in rod-like micelles consisting of two parallel aligned double helices.

reactions.[9] Furthermore, the influence of hybridizing the ss DNA corona of the micelles with different sequences was investigated.[10] It turned out that hybridization with short sequences that are complementary to the corona does not change the structural properties of the micelles. However, when long DNA sequences that encode several times the complementary sequence of the corona were employed for hybridization, highly uniform rod-like aggregates consisting of two parallel aligned DNA double helices were formed (Figure 1).

Within this contribution we investigate the cellular uptake of DNA block copolymer aggregates with a ss and double stranded (ds) DNA corona as well as different shapes. For spherical block copolymer micelles the influence of several physical parameters such as size and surface charge on the entry into cells were investigated. These results suggest that it is also worthwhile to explore the morphology of the aggregates as an important structural feature.

Experimental Part

Preparation of Caco-2 Monolayers

Caco-2 cells (passage number 45) were cultured at 37 °C in an atmosphere of 5% CO_2 and 90% relative humidity in 75 cm^2 cell culture flasks containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids, 100 $U \cdot mL^{-1}$ penicillin, and 100 $\mu g \cdot mL^{-1}$ streptomycin. The cells were routinely split and seeded into 6-well plates (Nunclon™ Multidishes, Life Technologies GmbH, Karlsruhe, Germany) with 800.000 cells per well. The medium was changed three times a week. The development of the monolayers was examined under the microscope until the 21st day.

Cytotoxicity Assay

Prior to the uptake experiments, the cytotoxicity of the nanoparticles was assessed using XTT *in vitro* toxicology assay kit following the procedure of the manufacturer (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Cellular Uptake of Nanoparticles

For the uptake studies that were carried out on day 21, the growth medium was removed and the Caco-2 monolayer in each well was washed twice with Hank's balanced salt solution (HBSS) containing 5 $mmol \cdot L^{-1}$ HEPES adjusted to pH = 7.4. The wells were filled and incubated with 2 mL of 300 $\mu g \cdot mL^{-1}$ Alexa488-labeled DNA-*block*-poly(propylene) oxide (DNA-*b*-PPO) micelles. After 3 h incubation at 37 °C the medium was discarded and cells were washed five times with ice-cold buffer. The cell monolayers were then solubilized with 700 μL of 1.25×10^{-3} M NaOH. Then the suspension was centrifuged to remove the cell components and the fluorescence of the supernatant was measured.

Results and Discussion

An amphiphilic DNA block copolymer combining a 22 mer oligonucleotide (ODN) (sequence: 5′-CCTCGCTCTG CTAATCCTGTTA-3′) with a PPO segment ($\overline{M}_w = 6\,800$ $g \cdot mol^{-1}$) in a covalent fashion was produced by employing an automated grafting onto strategy on the solid support as described previously.[9] As the hydrophobic component PPO was selected to provide a polymer with proven biocompatibility toward different cell types when administered as a constituent component of amphiphilic block copolymer micelles.[11] ss micelles were obtained by dissolving DNA-*b*-PPO in buffer at pH 7.4 (HBSS containing 5 $mmol \cdot L^{-1}$ HEPES) and heating. ds DNA block copolymer aggregates were obtained by hybridization either with the complementary sequence T22 (sequence: 5′-TAACAGGATTAGCAGAGCGAGG-3′) or with an ODN T110 (sequence: 5′-$(TAACAGGATTAGCAGAGCGA\ GG)_5$-3′) five times encoding the complement of DNA-*b*-PPO resulting in spherical micelles with a ds corona or rod-like micelles consisting of two parallel aligned double helices, respectively (Figure 1). ss- and ds spherical micelles exhibited a radius of 5.6 ± 0.5 and 5.3 ± 0.5 nm, respectively. For the rod-like particles a length and width of 29.1 ± 6.5 and 3–4 nm was determined, respectively. The dimensions of the different nanoparticles were measured by fluorescence correlation spectroscopy and by scanning force microscopy as described previously.[10]

Prior to study the uptake of the nanoscopic aggregates, their cytotoxicity at a concentration of 300 $\mu g \cdot mL^{-1}$ was assessed using an XTT based toxicology assay. Caco-2 cells

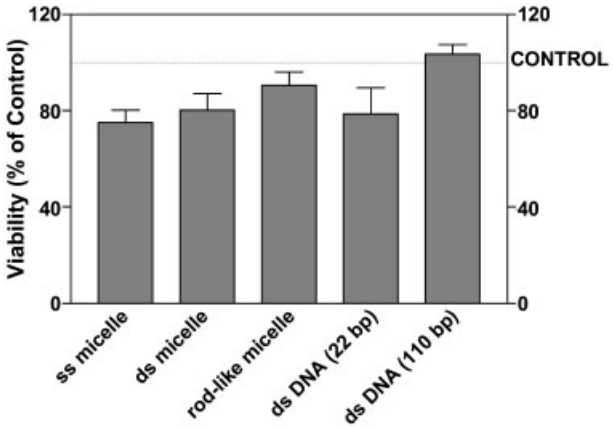

Figure 2. Viability of Caco-2 cells after incubation with different shaped nanoparticles and pristine DNAs. Control represents incubation without the addition of any DNA containing moiety. (Each bar represents the mean of three experiments ± SD).

(human colon adenocarcinoma) showed a high viability when incubated for 3 h with the different types of DNA block copolymer micelles as well as with DNA controls being non-modified by the organic polymer (Figure 2). Motivated by the non-toxic nature of the bioorganic hybrid materials, the uptake of the nanoparticles in the same cell line was investigated. For these experiments the micelles were labeled with a fluorophore. 5′-Alexa488-modified ODNs with the sequence of T22 or T110 were employed for hybridization with the micelles introducing the fluorescent reporter. Then the Caco-2 cells were incubated with the DNA block copolymer aggregates at a concentration of 300 $\mu g \cdot mL^{-1}$ for 3 h. Similar conditions have been employed to study the uptake of polymer functionalized ODNs[12] and block copolymer aggregates.[7,13] The internalization of the nanoparticles was investigated by confocal laser scanning microscopy (CLSM) and after lyzing the cells by fluorescence spectroscopy. The latter method allows comparative quantification of the uptake of the different DNA block copolymer aggregates. It turned out that the rod-like particles were internalized 12 times more efficiently than the spherical particles that were taken up similarly. The uptake of the pristine DNA controls was significantly less than for the micelle architectures (Figure 3).

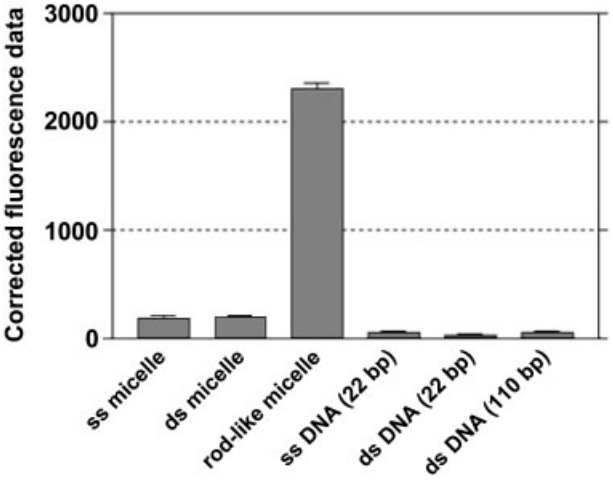

Figure 3. The internalization of the nanoparticles investigated by fluorescence spectroscopy after lyzing the cells. The micellar aggregates were compared with the pristine DNA. (Each bar represents the mean of three experiments ± SD).

CLSM has proven to be a powerful tool for acquiring high resolution images, 3-D reconstructions and visualization of internalization of nanoparticles.[14] The fluorescence microscopy images show that the nanoparticles were distributed homogenously inside the cells and did not just adsorb to the surface (Figure 4). No distinct patterns of subcellular staining were observed. In the case of the spherical micelles CLSM revealed a different degree of uptake among the cells. While some of the cells were stained others did only show weak fluorescence. This may be explained by the heterogeneous population of Caco-2 cells that leads to different uptake behaviors of the cells in the same population.[15,16] The uptake of block copolymer aggregates has been studied intensively in the context of drug- and gene delivery. However, these systems consisted exclusively of spherical nanosized objects; the internalization of rod-like block copolymer micelles has never been investigated. The uptake of rod-like nanoparticles was demonstrated for carbon nanotubes.[17,18] The only comparative study where nanoobjects of different shape were employed deals with inorganic nanoparticles.[19] These experiments revealed lower uptake of rod-shaped Au-nanoparticles compared to the spherical counterparts. However, the Au-nanoparticles of different geometries varied in surface functionalization and the rod-shaped particles were contaminated with non-rod-shaped by-products. In the experiments presented here contrary uptake behavior for DNA block copolymer nanoparticles was observed. Rod-like particles were internalized more efficiently than spherical particles. It should be pointed out here that the structures of the block copolymer aggregates were well defined. A possible explanation for the shape-dependent uptake might be different uptake processes for nanoparticles with varying geometries. Since in the rod-like particles the hydrophobic PPO blocks that could interact with the cell membrane are less shielded than in the spherical particles, adsorptive endocytosis might play a major role. In contrast the spherical micelles with the hydrophobic PPO buried in their interior might be taken up by fluid phase pinocytosis due to electrostatic repulsion of the negatively charged micelle corona and the cell surface as suggested for ODN-*block*-poly[(D,L-lactic acid)-*co*-(glycolic acid)] (PLGA) micelles.[7] The fact that the DNA block copolymer micelles were internalized more

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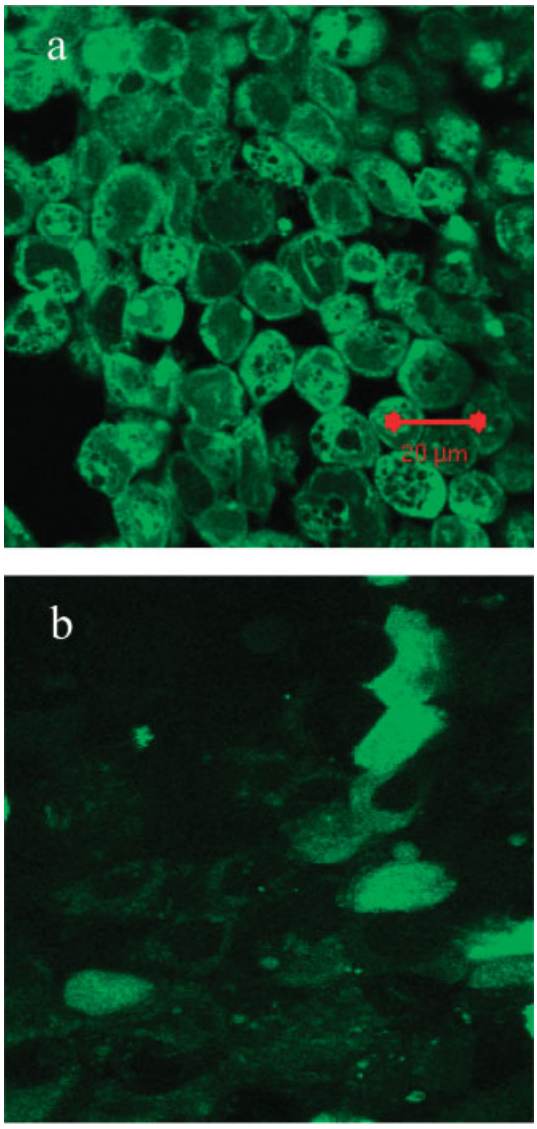

Figure 4. CLSM image of the Caco-2 cells incubated with fluorescently labeled (a) rod-like and (b) spherical micelles.

efficiently than the pristine DNA controls is in agreement with the literature and was also observed in the uptake studies of ODN-*b*-PLGA aggregates.[7]

Conclusion

In summary, the cellular uptake of DNA block copolymer aggregates with different shapes was investigated. It was found that rod-like nanoparticles were significantly more internalized than spherical particles, although, both types of self-assembled structures were built up from the same components. In future work, the uptake mechanism of these nanoobjects will be elucidated in more detail.

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